Definition of Interferon γ-response Elements in a Novel Human Fcγ Receptor Gene (FcγRIb) and Characterization of the Gene Structure

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Summary

The human FcγRI (CD64) is a high affinity receptor for the Fc portion of immunoglobulin (Ig), and its constitutively low expression on the cell surface of monocyte/macrophage and neutrophils is selectively upregulated by interferon γ (IFN-γ) treatment (Perussia, B., E. T. Dayton, R. Lazarus, V. Fanning, and G. Trinchieri. 1983. *J. Exp. Med.* 158:1092). Three distinct cDNAs have been cloned and code for proteins that predict three extracellular Ig-like domains (Allen, J. M., and B. Seed. 1989. *Science [Wash. DC].* 243:378). Several differences in the coding region of these cDNAs suggest that in addition to polymorphic differences a second FcγRI gene could possibly exist. This alternative FcγRI gene (FcγRIb) was defined by the lack of a genomic HindIII restriction site (van der Winkel, J. G. J., L. U. Ernst, C. L. Anderson, and I. M. Chiu. 1991. *J. Biol. Chem.* 266:13449). We describe the characterization a second gene (FcγRIb) that has a termination codon in the third extracellular domain and therefore predicts a soluble form of the receptor. We also define two distinct IFN-γ-responsive regions in the 5′ flanking sequence of FcγRIb that resemble motifs that have been defined in the class II major histocompatibility complex promoter. The FcγRIb promoter does not possess canonical TATA or CCAAT boxes, but does possess a palindromic motif that closely resembles the initiator sequence identified in the terminal deoxynucleotidyl transferase/human leukocyte IFN/adeno-associated virus type II P5 gene promoters (Smale, S. T., and D. Baltimore. 1989. *Cell.* 57:103; Seto, E., Y. Shi, and T. Shenk. 1991. *Nature [Lond.].* 354:241; Roy, A. L., M. Meisterernst, P. Pognonec, and R. C. Roeder. 1991. *Nature [Lond.].* 354:245) virus type II P5 gene promoters raising interesting questions as to its role in the basal and myeloid-specific transcription of this gene.

Treatment of mammalian cells with species specific IFNs (α, β, and γ) induces shared and distinct cellular responses that result from induction or repression of selected genes (1). IFN-α and -β are products of all cells, share a common receptor, and exert similar effects on cells. IFN-γ, a product of activated T cells and NK cells, has a distinct cell surface receptor and induces unique effects as well as responses shared with IFN-α and -β (reviewed in reference 2). In vitro and in vivo administration of IFN-γ indicates that its predominant physiological role is to modulate immune functions. In particular, IFN-γ, but not IFN-α and -β, induces monocyte differentiation, and IFN-γ is the predominant macrophage-activating factor (3, 4). As such, myeloid cells must have sets of genes whose expression is regulated in response to IFN-γ treatment. The net effect of these IFN-γ-dependent pleiotypic changes is that the treated phagocytes display an activated phenotype (5). Macrophages activated by IFN-γ treatment downregulate mannose receptors and collagenase secretion, and upregulate expression of components of the NADPH oxidase, IL-1, IL-8, TNF, IP10, monokine induced by IFN-γ (MIG),[1] the MHC class I and II antigens, and Fc receptors (6–13).

Responses of genes that are regulated by IFN-α/β appear to be mediated by a conserved motif, termed the IFN-stimulated response element (ISRE), located within their regulatory regions (1, 4, 14, 15). Interestingly, transcriptional upregulation of genes containing the ISRE by IFN-α/β are augmented by IFN-γ. ISGF3 is a positive multimeric trans-activating factor that targets the ISRE by translocating independent subunits from the cytoplasm to the nucleus upon IFN-α treatment. One component, ISGP3γ, appears essen-

[1] *Abbreviations used in this paper:* ISRE, IFN-stimulated response element; MIG, monokine induced by IFN-γ.

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tial for DNA binding and its synthesis is upregulated after treatment with IFN-γ (16). Although less well characterized, a similar complex interaction between multiple factors is likely to account for the transcriptional control of genes that are exclusively upregulated by IFN-γ. The best studied of these genes are the MHC class II genes whose promoters contain a series of conserved *cis*-acting elements termed X, Y, and H box and IFN-γ response element (γ-IRE) (reviewed in reference 17). A number of independent studies indicate that IFN-γ responsiveness of this region cannot be accounted for by a DNA binding protein with one precise element. Our focus is to examine the regions that determine IFN-γ responsiveness in the context of a myeloid cell so as to gain insight into the molecular mechanisms that underlie the role of IFN-γ as a macrophage-activating factor. To this end, the regulation of the human FcγRI gene is an attractive candidate as its expression is restricted to myeloid cells and is specifically upregulated by IFN-γ. IFN-γ treatment alone results in a rapid increase in transcription of the FcγRI gene that results in up to a 20-fold induction of mRNA levels and expression of hundreds of thousands of cell surface receptors (reviewed in reference 18). The study of the regulation of the FcγRI gene should therefore provide insights into defining *cis*-acting elements that confer myeloid specificity as well as definition of enhancers that target selective induction by IFN-γ.

FcγRI is a member of a family of membrane receptors that recognize the Fc domain of Ig. The characterization of Fc receptors by molecular cloning has detailed a complex family of molecules that have a wide cellular distribution and a considerable structural heterogeneity that defines the diverse cellular responses triggered by ligation of Fc receptors (18). FcγRI has a third Ig-like intracellular domain that determines its unique ability among Fc receptors to bind the monomeric Ig, and hence its designation as a high affinity Fc receptor. Three distinct cDNAs predict almost identical glycoproteins in their extracellular and transmembrane regions but differ in their cytoplasmic tails (19). Recent reports have detailed the genomic organization of the FcγRI gene (20, 21) and provided evidence to support the existence of a second gene as defined by a difference in a HindIII restriction fragment length (20).

In this study we provide a detailed analysis of the genomic organization of the putative second FcγRI gene (FcγRIb), which lacks HindIII in the second intron site due to a point mutation. In addition, a termination codon in the third extracellular domain and an insertion of a single amino acid appear to be the major points that distinguish this as a second gene. We have also mapped the regions in the FcγRI promoter that confer IFN-γ responsiveness and myeloid specificity. The *cis*-acting elements share some homology with defined IFN-γ elements found in MHC class II promoters as well as having unique properties.

Materials and Methods

Cells and Cell Cultures. U937, THP-1, Raw 264.7, and Hela cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in RPMI supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, and gentamycin (complete medium). All media and tissue cultures were scrupulously monitored to ensure endotoxin-free conditions.

Isolation of the 5' Flanking Region of the FcγRI Gene. A human genomic library was screened by plaque hybridization using the 5' portion of FcγRI cDNA (−27 to +157; numbering from the ATG) as the probe. After prehybridization in 6× SSC, 5× Denhardt's, 0.1% SDS for 2 h at 68°C, the random primed labeled probe (100,000 cpm/ml) was added and hybridization was performed at the same temperature for 12 h. Filters were washed serially in 2× SSC, 0.1% SDS for 30 min at 68°C, with 0.2× SSC, 0.1% SDS for an additional 30 min at 68°C, and with 0.2× SSC, 0.1% SDS for an additional 30 min at 68°C, followed by autoradiography for 12 h. Positive plaques were picked and rescreened for an additional two rounds, after which DNA of the positive phages was prepared, restriction digested, and analyzed on Southern blot prehybridized in 6× SSC, 1 × Denhardt's, 0.5% SDS, 0.05% sodium pyrophosphate at 37°C for 1 h and hybridized at the same temperature for 12 h in the same solution containing 10^6 cpm/ml of a 5' end-labeled oligonucleotide primer (−7 to −26; representing the 5' end of the cDNA).

Plasmid Construction. The PEcoR143 vector was created by subcloning the 4.3-kb EcoRI genomic fragment, representing the 5' exon and the 5' flanking region, into the EcoRI site of pUC9 vector. For the SV40 heterologous promoter CAT constructs, PEcoR143 was used as template to generate fragments synthesized by the PCR from appropriate oligonucleotide primers, to which were added sequences for BglII restriction endonucleases. The amplified fragment was then cloned into the BglII site of the reporter plasmid pCAT (Promega Biotec, Madison, WI), which contains the bacterial chlorophenicol acetyl transferase reporter gene downstream of the SV40 basal promoter element.

Construction of the TKCAT and Promoterless CAT Constructs. PEcoR143 was used as a template to generate progressively deleted fragments of the 5' flanking region spanning −242 to −5 by the PCR using the following primers: (*a*) NotI-FcPCR5b (189 to −160) + Xba-FcPCR3 (−28 to −7) = −189 to −7; (*b*) NotI-FcPCR5c (−128 to −99) + XbaI−FcPCR3 (−28 to −7) = −128 to −7; (*c*) NotI-FcPCR5d (−78 to −49) + XbaI-FcPCR3 (−28 to −7) = −78 to −7; (*d*) NotI-FcPCR5b (−189 to −160) + XbaI-GRR2 (−154 to −119) = −189 to −119; (*e*) BamHI-GRRa (−169 to −141) + BamHI-GRRb (−158 to −128) = −169 to −128; (*f*) NotI-FcPCR5c (128 to −99) + XbaI-gI-PCR1 (−102 to −74) = −128 to −74; (*g*) BamHI-GRRa (−169 to −141) + XbaI-GRR2 (−159 to −119) = −169 to −119.

The fragments generated in reactions *a–d* were ligated into the NotI and XbaI sites of pBRAMS cat1 (22) to generate CAT constructs in which the CAT gene is under control of the endogenous CD64 5' flanking region promoter (see Fig. 5 *B*).

Fragments generated in reactions *d–h* were ligated into either NotI/XbaI, BamHI, or NotI (blunted)/XbaI (in the case of "h") sites upstream of the TK promoter in the vector pBRAMS cat2.

Cell Transfection. U937 or THP-1 cells were grown as suspension cultures in 250-ml flasks. 5 × 10^6 cells were transfected with 20 μg of DNA using the DEAE-dextran method (23). Briefly, 40 μg DNA was combined with 400 μg/ml of DEAE-dextran in a final volume of 1 ml of media containing 10% Nu-Serum. After 5 min at room temperature, the mixture was added to a 1-ml cell suspension (10^7 cells/ml) in media containing 10% Nu-Serum and incubated for 2 h at 37°C, 5% CO_2-air. The cells were then washed once in Dulbecco's PBS and shocked for 3 min in 10% DMSO in HBSS (5 mM KCl, 0.4 mM KH_2PO_4, 140 mM NaCl,

0.3 mM Na_2HPO_4-$12H_2O$, 5.5 mM glucose, 5 mM EDTA) at 37°C. The DMSO was then diluted by addition of excess medium to the cells, washed twice with PBS followed by resuspension in 20 ml complete medium, and divided into two equal aliquots of 10 ml each. Each aliquot was then grown for 48 h at 37°C/5% CO_2-air before induction of one aliquot with 300 U/ml of recombinant human IFN-γ at 37°C for 12 h. The other aliquot served as the noninduced control.

Hela and RAW 264.7 cells were plated at a density of 10^6 cells/100-mm petri dish on the evening before the day of transfection. The following morning each 100-mm dish was washed twice with PBS. Each dish was then incubated with a mixture of 20 μg DNA, 200 μg/ml DEAE-dextran in a final volume of 3.5 ml of RPMI 1640 containing 10% Nu-Serum. The dishes were incubated for 2–4 h at 37°C/5% CO_2-air. The monolayers were washed once with PBS and shocked for 3 min at 37°C with 3 ml of 10% DMSO in HBSS. The DMSO was then diluted by an addition of excess medium to the cells, washed gently once with PBS, followed by an addition of excess medium to the cells, washed gently once with PBS, followed by an addition of 10 ml complete medium. The dishes were incubated overnight at 37°C/5% CO_2-air. Each dish was then split by limited trypsin-EDTA treatment and replated in two equal aliquots in 60-mm dishes and reincubated for an additional 24 h before stimulation of one subset with 300–400 U/ml of recombinant human or murine IFN-γ for 12 h at 37°C. The other subset served as the noninduced control.

CAT Assays. Cells were harvested and CAT assays were performed as follows. Adherent cells were washed three times with PBS and then incubated 5 min at room temperature in 1 ml of TEN buffer (40 mM Tris-HCl pH 7.5, 1 mM EDTA, 15 mM NACl). The cells were then scraped off with a rubber policeman, and the suspension was spun in an Eppendorf microfuge for 10 min at 4°C at 14,000 rpm. The cell pellet was resuspended in 150 μl of 0.2 M Tris-HCl, pH 8, and subjected to three freeze-thaw cycles with vortexing after each thaw cycle. After heating at 60°C for 10 min, the extract was spun for 10 min at 4°C and the supernatant was transferred to a fresh tube. CAT activity was performed on 50 μl of the other by extraction, using *n*-butyryl coenzyme and xylene extraction as described in the CAT enzyme assay system protocol of Promega Biotech followed by liquid scintillation counting or by separation on TLC. Activities were adjusted for differences in the protein concentration of the extracts. Nonadherent cells were washed three times in PBS. The cell pellet was then resuspended in 1 ml of TEN buffer and the suspension was transferred to a microfuge tube and incubated at room temperature for 5 min. The subsequent steps were the same as for adherent cells.

Results

FcγRIb Gene. While considerable structural differences characterized FcγRII and FcγRIII gene families, FcγRI (CD64) has been considered a product of a single gene though three distinct cDNAs had been described. We isolated genomic clones by using a radiolabeled FcγRI cDNA (p130) as a probe. The genomic structure indicates six exons (Fig. 1) that predict a polypeptide that differs from the predicted products indicated by the three published FcγRI cDNAs. Although each extracellular (EC) domain is encoded by a distinct exon, as would be predicted for members of the Ig superfamily, there is a termination codon at bp705 in EC domain 3 that would predict a soluble form of the receptor. We have also noted that at bp565, nucleotide TG does not appear in the cDNAs but is replaced by a 6-bp ACAATA sequence in the genomic clone. The net result is that the gene sequence predicts an extra in-frame amino acid, Gln. Further differences in the genomic sequence of FcγRI and FcγRIb are reflected at 6 bp within the 5′ boundary of the fifth intron, as underlined in Table 1, and several point mutations that are highlighted in Table 2. The entire gene is interrupted by five introns that demarcate six exons (shown in Fig. 1 and Table 1). The first exon begins at the transcription initiation sites (see below) and encompasses the translation start site and ·he putative signal peptide. Next is a small 21-bp exon followed by three extracellular Ig domain exons. The 3′ UT region is encoded by the sixth exon.

The p98/X2 cDNA predicts a polypeptide that has a shorter cytoplasmic tail with a divergent sequence. We have been unable to identify the exon(s) coding for this divergent sequence. It is possible that this variant form could arise as a result of alternative splicing and that a further 3′ exon(s) encodes the 12 divergent amino acids present in the shorter cytoplasmic tail of clone p98/X2.

The FcγRI Upstream Regulatory Region. We next set out to determine whether FcγRI mRNA was upregulated as a specific response to IFN-γ treatment (reviewed in reference 18). Fig. 3 *a* shows that overnight treatment of the myelomonocytic cell line U937 with IFN-γ but not IFN-α resulted in an induction of two distinct mRNAs of ∼1.7 and ∼1.2 kb, respectively. It seems likely these mRNAs rep-

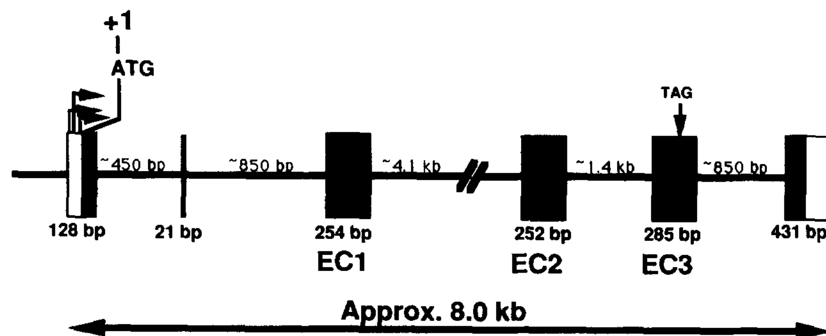

Figure 1. Human FcγRIb genomic organization. Open boxes represent untranslated regions of exons; filled area represent coding region. The numbers above the boxes indicate the size of exons, while those below indicate the size of introns. Ec, external domain; CYT, cytoplasmic tail.

Table 1. *Human FcRI Gene: Exon-Intron Boundaries*

Intron	5′ boundary	Intron length	3′ boundary
		kb	
	67		68
I	CTC CTT TGG G	gtaagttggact......... ∿0.45 ....gatctcttgcag	TT CCA GTT GAT
	Leu Leu Trp V		al Pro Val Asp
	88		89
II	GGG CAA GTG G	gtgagtgatctc......... ∿0.85 ....atctccaagtag	AC ACC ACA AAA
	Gly Gln Val A		sp Thr Thr Lys
	343		344
III	ATC CAC AGA G	gtaattatgact......... ∿4.10 ....catattttcag	GC TGG CTA CTA
	Ile His Arg G		ly Trp Leu Leu
	595		596
IV	ACT GTG AAA G	gtattgtattgg......... ∿1.40 ....ctttctccttag	AG CTA TTT CCA
	Thr Val Lys G		lu Leu Phe Pro
	880		881
V	CAA GTG CTT G	gtgagaatgacg........ ∿0.85 ....tttctgtttcag	GC CTC CGG TTA

The underlined nucleotide (no. 887) surrounding the 3′ boundary of intron V represents a substitution from A (cDNA) to G (gene) resulting in a change in the amino acid encoded from Gln to Arg (See Table 2). The underlined sequences are different from the published FcRI gene sequence (van de Winkel et al., 1991). The numbering of the cDNA nucleotide sequence corresponds to FcRI cDNA Genbank accession no. M21091.

resent inducible transcripts from FcγRIa and the FcγRIb gene. The induction of FcγRI by IFN-γ has been previously shown to be mediated at the level of transcription (21). In addition, it appears that TNF-α alone does not have an effect on FcγRI expression and does not augment the IFN-γ-dependent induction (Fig. 3 *b*). This induction occurs in the presence of cyclohexamide and IFN-γ but not with cyclohexamide alone, suggesting that de novo protein synthesis is not required and that it represents an immediate early response to IFN-γ (Fig. 3 *b*). Once we had confirmed that induction of FcγRI genes appeared to be IFN-γ specific, we set out to define the *cis*-acting elements that confer IFN-γ responsiveness. The sequence of the upstream region was determined (Fig. 4) and the transcription start site(s) defined. We isolated RNA from U937 myelomonocytic cells (before and after treatment) with IFN-γ. This mRNA was hybridized to a radiolabeled probe that spanned from +14 to −799 bp and digested with S1 nuclease. Three protective bands that mapped to positions −97, −83, and −82 were detected upstream from the ATG (+1), when mRNA from IFN-γ-treated cells was used as a template, but not with mRNA from untreated cells or tRNA (not shown). To confirm that these protective fragments corresponded to the transcription start site of FcγRIb, a primer extension analysis was performed using an N-labeled oligonucleotide from +14 to −9 bp (not shown). Three extended fragments that were identical to those seen in the S1 protection assay were observed and are indicated in Fig. 4. It should be noted that the transcriptional start sites for the FcγRIb gene are in a similar region, but are not identical to those described for the FcγRIa gene (20, 21). Our results indicate that FcγRI transcription can be initiated at three independent start sites and that there are no canonical, TATA, or CCAAT boxes in the 5′ region flanking of the transcription start sites. Due to the absence of a single defined major transcription initiation site, the numbering of nucleotide positions in the 5′ flanking region was assigned on the basis of the first nucleotide of the translation start codon as +1.

Definition of IFN-γ Response Elements. Our next goal was to explore the possibility of whether the IFN-γ response elements reside in the upstream flanking region of FcγRIb gene. The region from −689 to −7 derived from the 4.3-kb EcoRI genomic fragment, as described in Materials and Methods, was cloned into a BglII site of the promoterless CAT vector, pBRAMS cat I (described in Materials and Methods). When this construction was transfected into the human myelomonocytic cell line, U937, followed by overnight treatment with recombinant IFN-γ, a 14-fold induction of CAT activity over background was observed (not shown). To better define the gamma response region (GRR), a series of deletion constructions were prepared and defined fragments cloned into the pBRAMS cat I vector. 14-fold IFN-γ-inducible activity was observed with a fragment from −189 to −7. Further deletion to bp 128, i.e., generating a fragment from −128 to −7, resulted in a reduction in activity to sevenfold induction. The induction of the reported gene for both these constructs was observed only in myeloid cells and not in Hela cells. However, a fragment from −79 to −7 was not active in either myeloid or Hela cells, suggesting that the IFN-γ

Table 2. *Mutations in the Human FcRI Gene*

Type	Nucleotide			Amino acid	
	Position	From	To	From	To
Substitutions:					
Coding regions	109	T	A	Ser	Thr
	195	T	C	–	–*
	380	C	T	Thr	Met
	706	C	T	Gln	Termination
Noncoding regions	1	G	T		
	851	G	A		
	887	A	G		
	1006	G	A		
	1049	C	T		
	1174	G	T		
	1217	G	A		
	1279	G	A		
Deletion & Insertions					

571									
cDNA	GCA	GGA	ATA	TCT	*GT*C	ACT	GTG	AAA	
	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	
Gene	GCA	GGA	ATA	TC**A**	**CAA**	T**A**C	ACT	GTG	AAA
	Ala	Gly	Ile	Ser	Gln	Tyr	Thr	Val	Lys

The nucleotides italicized and underlined in the cDNA sequence were found deleted in the gene. The nucleotides in bold letters and underlined were found to be inserted in the genomic sequence.
* Silent substitution (Ser).

response regions resided between upstream fragment spanning from −189 to −128 and the downstream region between −128 and −79.

We next set out to better define the sequences between −189 and −79 that would be necessary and sufficient for IFN-γ responsiveness in the context of a heterologous promoter so that we could assay expression in U937 and Hela cells. Our initial attempts were complicated by the use of a vector containing the SV40 promoter, as this appeared to silence the upstream IFN-γ response region. A construct from −189 to −7 in the context of the SV40 promoter resulted in a fivefold induction after IFN-γ treatment. A further series of deletion constructs in the context of the SV40 promoter suggested that sequences contained within −104 to −78 were necessary and sufficient to account for this modest induction after IFN-γ treatment (results not shown). On the advice of Pearse et al. (21), we refocused our attention on the upstream region −169 to −128, as this region has been shown by this group to confer IFN-γ responsiveness in the context of the basal promoter and a thymidine kinase promoter. As it appeared that this region had been silenced in the context of the SV40 promoter, we prepared a construct containing an upstream sequence from −169 to −128 in pBRAMS cat 2 vector that contains the heterologous thymidine kinase promoter. We were not able to detect a 10-fold induction in reporter gene activity in U937 cells after IFN-γ treatment.

In our earlier studies we had noted a motif RGAAAAG that resembles a general IFN responsive element, RGAAACG (24), in both the −104 to −78 as well as immediately upstream from −169. We therefore prepared a construct from −189 to −119 (nine extra downstream bases were included to maintain the correct context) in pBRAMS cat 2, and found that this construct induced 20–50-fold induction of CAT activity after treatment with IFN-γ of both Hela and U937 cells. These results suggested that this upstream region was necessary and sufficient to account for IFN-γ responsiveness and, while the downstream region was active out of context, it probably is not physiologically relevant as inclusion of this region does not confer additional activity.

To better define basal promoter activity, we next prepared a construct in which −189 to −119, i.e., the IFN-γ response region was inserted into the promoterless CAT vector, and recombinant plasmid was transfected into U937 and Hela cells. The results indicate that no inducible activity was observed, suggesting that the basal myeloid promoter most likely resides between −119 and −7 (Fig. 5 *A*). It should be noted that when the same upstream fragment was placed in the context of the heterologous TK promoter, maximum IFN-γ-inducible activity was observed in both U937 and Hela cells (Fig. 5 *B*). Of note is that the region containing sequences from −128 to −7 resulted in a sevenfold induction after treatment of U937 cells with IFN-γ but not Hela cells. However, when this region was placed in the context of the heterologous TK promoter, it was active in both myeloid and Hela cells. These results suggest that the basal myeloid promoter resides in the region from −128 to −7. Careful scrutiny of this region revealed that there is a 16-bp region that is precisely conserved in the 5′ upstream region of the mouse αFcγRIII gene (Fig. 6, *A* and *B*) as well as a potential Pu box motif. The presence of a putative Pu box (Fig. 6) is relevant as its recognition site for the Pu.1 protein, a transcriptional activator expressed in macrophages B cells (25). From these findings, we have designated this region the "PIE region" for Pu.1 and IFN-γ elements spanning −104 to −84.

Discussion

Treatment of myeloid cells with IFN-γ results in activation of effector mechanisms that include upregulation of respiratory burst activity and 20-fold induction of FcγRI expression (reviewed in reference 18). The study of FcγRI expression therefore provides an opportunity to explore *cis*-acting elements that confer baseline myeloid specificity as well as induction by IFN-γ. In this report we have characterized the gene structure of a second FcγRIb gene that predicts a soluble form of the receptor. We have also defined *cis*-acting elements that appear to be necessary and sufficient for IFN-γ responsiveness. Not surprisingly, these *cis*-acting elements most likely serve as recognition sites for several nuclear proteins whose interactions are probably critical in baseline myeloid

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gaattcatcctagtttccatttagctctctttctctcttaccacaatacctaaaaatctttttatggggttacaact
ctgtctcaggagccacctcagtttggtggcacagagctccccacttgtactctgaaactcttttcttgaagatattg
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GATTTCACTGCTCCCACCAGCTTGGAGACAACATGTGGTTCTTGACAACTCTGCTCCTTTGGGgtaagttggactca
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....(4100bp)...................cgtatacatacattttgggttcatatttttcagGCTGGCTACTAC
TGCAGGTCTCCAGCAGAGTCTTCATGGAAGGAGAACCTCTGGCCTTGAGGTGTCATGCGTGGAAGGATAAGCTGGTG
TACAATGTGCTTTACTATCGAAATGGCAAAGCCTTTAAGTTTTTCCACTGGAATTCTAACCTCACCATTCTGAAAAC
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ACACTGTGAAAGgtattgtattggaatagtcatagaactgatagtccctcccccctgaggaccatcataaatattcta
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CCACAGAGGATGGAAATGTCCTTAAGCACAGCCCTGAGTTGGAGCTTCAAGTGCTTGgtgagaatgacgggaagcca
ctggcacagaagaagggactcccttatctcccatgggactgaggtttgttcaaggg.....................
(850bp)...........tctggtctctaaatagtatctcttctctttgtctttttctgtttcagGCCTCCGGTTAC
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ACAATACGTAAAGAACTGAAAAGAAAGAAAAAGTGGAATTTAGAAATCTCTTTGGATTCTGGTCATGAGAAGAAGGT
AATTTCCAGCCTTCAAGAAGACAGACATTTAGAAGAAGAGCTGAAATGTCAGGAACAAAAAGAAGAACAGCTGCAGG
AAGGGGTGCACCGGAAGGAGCCCCAGGGGGCCACGTAGCAGCGGCTCAGTTGGTGGCCATCGATCTGGACCGTCCCC
TGCCCACTTGCTCCCCATGAGCACTGCGTACAAACATCCAAAAGTTCAACAACACCAGAACTGTGTGTCTCATGGTA
TATAACTCTTAAAGCAAATAAATGAACTGACTTCaactgggatacatttggaaatgtggtcatcaaagatgacttga
aatgaggcctactctaaagaattcgagctc
```

Figure 2. Nucleotide sequence of the FcγRIb gene sequence. Exons are in upper case and partial intron sequences are in lower case. The underlined nucleotides represent substitutions, whereas italicized and underlined sequences represent insertions. The mutated HindIII site in the second intron is double underlined.

expression as well as IFN-γ-inducible expression of the FcγRI gene family.

Initially, FcγRI was defined by several mAbs and, subsequently, three homologous cDNAs have been characterized. The ectodomain of each cDNA, designated p130, p98/X2, and p90, all predict proteins with a third extracellular Ig domain, a unique feature of FcγRI (19). Several polymorphisms were featured in the original report of these cDNAs in which the p90/p130 transcripts were thought to be dominant. The

Figure 3. Northern blot analysis of mRNA derived from U937 cells. (*a*) NI indicates no induction. 1,000 U IFN-α treatment for 4 h. 300 U IFN-γ treatment for 4 h was sufficient to induce two specific FcγRI transcripts of 1.7 and 1.2 kb, respectively. (*b*) Indicates that induction of stable FcγRI mRNA transcripts is IFN-γ dependent and does not occur after treatment with recombinant TNF. TNF does not alter IFN-γ-dependent induction. Cyclohexamide treatment does not superinduce mRNA transcripts nor does it blunt IFN-γ inducibility.

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-800 GAATTCATCCTAGTTTCCATTTAGCTCTCTTTCTCTCTTACCACAAT
-763 ACCTAAAAATCTTTTTATGGGGTTACAACTCTGTCTCAGGAGCCAC
-704 CTCAGTTTGGTGGCACAGAGCTCCCCACTTGTACTCTGAAACTCTTT
-660 TCTTGAAGATATTGTTATCCATGTGCATGAGTTATTTTTCTTCTCCA
-613 TAGCATCCACATTAAACAATTAATTACCTTGGATTTAGCCAAAAT
-568 AAACACTCCAAATTCATGATGTCTTTCAGGAAGAGAGTTTGTTTTC
-522 TTTTTTAACCAAAATGGGAGATTGATCCTGTCTGTGTCCCCTAGAG
-478 GAGCAGAGGCTGGTTTGTATTAGGGCAGCTCTTGTAGATGAGTAAC
-430 TTTTCCCATGGCCTCATAGAGGCTGATATAGATACTTCTGGATTCA
-384 AATGATTGTTTGGAGGCATTAGCAGGCATTGAACCAATTATAAAG
-349 AGTGAGGTTTTGCCATATTCTAACCGTAAGAAACAGAAACGGTAG
-294 AGGGTAAAAATAGAATAGAATGTCAACACAACTTTAGCCTTTTCC
            -242
-249 CTGGATTCTGAGAGTTCATAACCTGAAAATCAGAGATTCAAACTGG
                -189                         -169
-203 GAGAGATGGGCTAACAGGTATGAGCATGGGAAAAGCATGTTTCAA
                                  128       -119
-158 GGATTTGAGATGTATTTCCCAGAAAAGGAACATGATGAAAATGGT
           -104        -97                -82 -83
-113 CAGAAAAGGCAATTTCCTTCCTCTTTTCTAATTTGGCTCTGGAGCCA
-66  CCAGCAGAACCTCTTCAATATCTTGCATGTTACAGATTTCACTGCTC
                -7      +1
-19  CCACCAGCTTGGAGACAAC ATG TGG TTC TTG ACA ACT CTG CTC
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Figure 4. Upstream region of FcγRIb gene. Filled arrows indicate transcription start sites as determined by primer extension and S1 nuclease analysis. +1 designates the translation start site, and the beginning of the open reading frame is underlined.

Figure 5. CAT analysis data of the FcγRI gene promoter/enhancer region. The fold increase in CAT gene expression after 12 h of stimulation with 300 U/ml of human recombinant IFN-γ is shown for the particular cell line transfected. The arrows indicate potential transcription initiation sites in the relevant constructs.

p98/X2 transcript has complex direct repeats in the 3′ region leading to speculation that this may represent a cDNA cloning artifact. The p90/p130 share identity up to the 19th amino acid of the cytoplasmic tail and thereafter diverge (19). The p90 transcripts predict another 12 amino acids vs. 42 extra amino acids in p130. This difference may be accounted for by alternative splicing and there appears to be a cryptic splice donor site at the point where the sequences diverge. The presence of multiple differences in the three transcripts isolated from the same patient led Allen and Seed (19) to raise the possibility for another FcγRI gene. Van der Winkel et al. (20) have provided very compelling evidence to support this conclusion based on a Southern blot analysis of genomic DNA from three randomly selected individuals. These results suggested the existence of a second FcγRI gene that lacks one of the two internal HindIII sites. The gene structure represents this second gene in that we show that the lack of the HindIII site is a result of a point mutation in the second intron (Fig. 2), as well as several other differences in the exon sequence. Our genomic analysis predicts insertion of an extra amino acid in exon 2 as a result of a GT deletion and ACAATA insertion (Table 2) in the genomic sequence, and therefore predicts a protein sequence of SQYTV genomic vs. SVTV

Figure 6. Sequence of promoter/enhancer region of the FcγRI gene. (*A*) The gamma response region (GRR) and the PIE region are identified within the open boxes. The putative sequence that appears to confer IFN-γ responsiveness is shown within the lightly stippled box, and its relationship to the X-box consensus motif of the class II MHC enhancer is shown (nonhomologous nucleotides are underlined). The potential Pu-box is shown within the darkly stippled box in the PIE region. The bent arrows at −82, −83, and −97 indicate transcription initiation sites. A 14-bp palindromic sequence (−80 to −67) that closely resembles the "initiator" (Inr) sequence present in the TdT, adeno-associated virus type d P5 promoter, and the human leukocyte IFN gene promoter is shown within incomplete boxes. A degenerate consensus sequence of the IFN-γ element is shown at the bottom of the figure. (*B*) Homology between the human FcγRI promoter region with the 5′ flanking region of the murine α FcγRIII gene. The PIE region of the human FcγRI promoter is bracketed.

for the cDNA. At position 706, a C to T change predicts a termination codon and therefore predicts a soluble form of the receptor. Northern blot analysis revealed two IFN-γ-inducible transcripts (Fig. 3), further supporting the existence of the alternative form of the gene. Several differences in nucleotide sequence of the FcγRIb gene and the p130 cDNA have been highlighted in Table 2. It seems likely that the diversity in the coding and the noncoding region represents differences between two highly homologous genes as well as polymorphism within these genes. In fact, recent findings by Ernst et al. (26) confirm the existence of this FcγRIb and describe the presence of a third FcγRI gene. It therefore appears that FcγRI, like the other Fc receptors, is part of a multi-gene family. The characterization of a soluble form of FcγRIb, its gene product, and its physiology role remain to be determined.

Our results indicate that sequences contained in the region from −189 to −119 are necessary and sufficient to confer IFN-γ responsiveness. Pearse et al. (21) have illustrated in the FcγRIa gene, promoter sequences from −169 to −128 as the GRR and have highlighted homologues with elements that are represented in the promoters of MHC class II genes that appear necessary for basal expression as well as IFN-γ responsiveness. These *cis*-acting elements include X, H and γ-IRE box motifs (17), and their precise role in mediating IFN-γ responsiveness in the context of the FcγRI genes remains to be determined, especially as our results indicate that the FcγRI gene family response to IFN-γ is cycloheximide insensitive (Fig. 3), whereas the IFN-γ-dependent upregulation of MHC class II genes requires new protein synthesis (17). Our results indicate the importance of another motif, RGAAAAG, that is represented in upstream region −189 to −169 and in downstream region −113 to −104, and −95 to −84, and its inclusion confers enhanced IFN-γ responsiveness (Fig. 6 *A*). Of interest is that this motif is similar to a general IFN response element, GAAACG, which is found in the promoters of IFN-responsive genes and appears as a *cis*-acting element of HSV immediate early genes (24). It is interesting to note that IFN-α treatment impeded transcription of HSV-1 IE genes, and we postulate that the squelching effects observed after IFN-γ treatment, which we observed in the context of the SV40 promoter, may occur by a similar mechanism.

It is likely that myeloid specificity is determined in large part by the basal promoter. In this regard, it is important to note that in the absence of a heterologous promoter, both the GRR and PIE regions function only in myeloid cells. The identification of a potential Pu box is relevant, as Pu.1 proteins appear to be important partly in determining myeloid-specific gene expression (25). The absence of canonical TATA and CAT boxes led us to define a 14-bp palindromic sequence beginning at position −80, 2 bp downstream from the most 3′ transcription start site (Fig. 6). The sequences closely resembles the initiator sequence that appears to be both necessary and sufficient for basal transcription of the terminal deoxynucleotidyl transferase gene (27), adeno-associated virus type 2 P5 promoter (28), and the human leukocyte IFN gene (hLeIF) (29). The initiator sequence in these genes has been shown to mediate transcription by binding to the YY1 protein, which appears to direct the transcription machinery in the absence of TATA elements (30, 31). The importance of the initiator element in basal and tissue-specific transcription of the FcγRI gene remains an open and interesting question.

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